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(54) Title: CLONING OF LFA-1

(57) Abstract

The invention features substantially pure recombinant β -subunit of a human glycoprotein concerned with cellular adhesion, or a biologically active fraction thereof, an analog thereof, or a fragment thereof composed of at least 10% of a contiguous sequence of the β -subunit; a cDNA sequence coding therefor; and a vector containing a DNA sequence coding therefor. The invention also features monoclonal antibodies raised against the recombinant β -subunit of human LFA-1. Methods of using the glycoprotein and analogs thereof and antibodies are also disclosed as is a nucleic acid molecular hybridization assay using DNA probes.

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CLONING OF LFA-1

Background of the Invention

The work described herein was performed with the aid of government funding, and the government therefore has certain rights in the invention. Specifically, the work was supported by N.I.H. grants CA 5 31798 and AI 05877.

This invention relates to cellular adhesion.

Cellular adhesion is a critical function for guiding migration and localization of cells, and for maintaining the integrity of the body. Receptors for 10 extracellular matrix components such as fibronectin, laminin, and vitronectin mediate cellular adhesion during morphogenesis and wound healing. In the immune system, regulatory networks require intimate cell-cell interaction among lymphocytes and antigen-presenting 15 accessory cells, and cell-mediated cytolysis involves direct contact between the effector cell and virally-infected or transformed target cells.

Leukocyte-endothelial interactions are important in leukocyte mobilization into inflammatory sites and in 20 lymphocyte recirculation. These cellular adhesion reactions are mediated in part by a family of structurally related glycoproteins, LFA-1, Mac-1, and p150,95, all of which share a common β -subunit (hereinafter referred to as the β -subunit of human 25 LFA-1). (Springer et al., 314 Nature 540, 1985; Springer et al., Ann. Rev. Immunol. Vol. 5, 1987; both hereby incorporated by reference).

Summary of the Invention

In general, the invention features a) 30 substantially pure recombinant β -subunit of a human glycoprotein concerned with cellular adhesion, or b) a biologically active fraction of this β -subunit, c) an

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analog of the β -subunit, or d) a fragment of the β -subunit, composed of at least 10% of a contiguous sequence of the β -subunit. The invention also features a cDNA sequence encoding the β -subunit; and a vector containing said cDNA sequence. By recombinant subunit is meant the polypeptide product of recombinant DNA encoding the β -subunit, i.e., the polypeptide expressed from DNA which is not in its naturally occurring location within a chromosome. By natural subunit is meant that subunit produced naturally *in vivo* from naturally occurring and located DNA. By analog is meant a polypeptide differing from the normal polypeptide by one or more amino acids, but having substantially the biological activity of the normal polypeptide. The invention also features any monoclonal antibody (MAb) raised against the recombinant β -subunit, a biologically active fraction, an analog, or a fragment thereof composed of at least 10%, preferably at least 80%, of a contiguous sequence of the β -subunit of a human glycoprotein.

The cDNA sequence encoding the LFA-1 β -subunit or a fragment thereof may be derived from any of the naturally occurring genes encoding it, or synthesized chemically. Variations in this sequence which do not alter the amino acid sequence of the resulting protein, or which do not significantly alter the biological activity of the protein, are also acceptable, and are within this invention.

Preferably the human glycoprotein is LFA-1, Mac-1 or p150,95.

As will be described in more detail below, the invention permits the diagnosis and treatment of a variety of human disease states.

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Other features and advantages of the invention will be apparent from the following description of the preferred embodiments thereof and from the claims.

Description of the Preferred Embodiments

5 The drawings are first briefly described.

Drawings

Fig. 1 is the DNA coding sequence of the β -subunit of LFA-1, Mac-1 and p150,95. Potential N-glycosylation sites are marked with triangles.

10 Fig. 2 is a comparison of the amino acid sequence predicted from the cDNA in Fig. 1, and the amino acid sequence derived from enzyme digests of the β -subunit of LFA-1. Ambiguous determinations of amino acids are bracketed. The code for amino acids is as
15 follows:

	Ala,	A	-alanine .
	Arg,	R	-arginine
	Asn,	N	-asparagine
	Asp,	D	-aspartic acid
20	Cys,	C	-cysteine
	Gln,	Q	-glutamine
	Glu,	E	-glutamic acid
	Gly,	G	-glycine
	His,	H	-histidine
25	Ile,	I	-isoleucine
	Leu,	L	-leucine
	Lys,	K	-lysine
	Met,	M	-methionine (start)
	Phe,	F	-phenylalanine
30	Pro,	P	-proline
	Ser,	S	-serine
	Thr,	T	-threonine

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Trp,	W	-tryptophan
Tyr,	Y	-tyrosine
Val,	V	-valine

Methods

5 In general, the β -subunit of any of the above described related glycoproteins is isolated by standard procedures and the amino acid sequence of at least a part of it determined. From this analysis a synthetic oligonucleotide probe, corresponding to the amino acid 10 sequence, is synthesized and used as a probe for a genomic or cDNA library containing a DNA sequence encoding the β -subunit. An example of this procedure is given below. One skilled in the art will realize that this represents only one of many methods which can be 15 used to achieve cloning of the gene encoding the LFA-1 β -subunit.

Purification of the β -Subunit

MAb's directed against the alpha subunits of p150,95, Mac-1, and LFA-1, were used to affinity purify 20 their respective proteins from three different sources. The p150,95 protein was purified from hairy cell leukemia spleens (Miller et al., 1986, 137 J. Immunol. 2891, hereby incorporated by reference); Mac-1 was purified from pooled human leukocytes (Miller et al., 25 supra); and LFA-1 was purified from the SKW3 T cell line using TS1/22 monoclonal antibody (Sanchez-Madrid et al. 1983, J. Exp. Med. 158:586, hereby incorporated by reference).

Preparative SDS-PAGE gels were run using the 30 method of Laemmli (Hunkapiller et al., 1983, Meth. Enzym. 91:227). 0.1 mM Na thioglycolate was added to the upper chamber to reduce the level of free radicals in the gel. Bands were visualized by soaking the gel

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for several minutes in 1 M KCl and then excised. The β -subunit was electroeluted using the apparatus and method described by Hunkapillar et al., supra. The purified protein was reduced with 2 mM DTT in the
5 presence of 2% SDS and alkylated with 5 mM iodoacetic acid in the dark. (In some cases, the protein was reduced and alkylated prior to running the preparative gel.)

Amino acid sequencing

10 The above samples were precipitated using four volumes of ethanol at -20°C for 16 hr, and the protein pellet redissolved in 30-50 ml of 0.1 M NH_4CO_3 containing 0.1 mM CaCl_2 and 0.1% Zwittergent 3-14 (Calbiochem, San Diego, CA). The sample was then
15 digested with 1% w/w trypsin for 6 hr at 37°C. At 2 and 4 hr during the incubation, additional trypsin (1% w/w) was added.

The tryptic peptides were resolved by reverse phase HPLC (Beckman Instruments) with a 0.4 X 15 cm C4
20 column (Vydac, Hesperig, CA), and eluted from a 2 hr linear gradient from 0 to 60% acetonitrile. 0.1% TFA was included in both the aqueous and organic solvents. The peaks were monitored at 214 and 280 nm and collected into 1.5 ml polypropylene tubes. The fractions were
25 concentrated to 30 ml or less on a speed-vac apparatus, and selected peptides subjected to sequence analysis using a gas phase microsequenator (Applied Biosystems, Foster City, CA).

Example: β -subunit of p150,95

30 p150,95 was affinity purified from the spleens of human patients with hairy cell leukemia using a monoclonal antibody specific for the alpha subunit (MW approx. 150,000, Miller et al., supra). Analysis of the

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purified protein by SDS-PAGE and silver staining revealed the characteristic alpha and beta subunit, with no significant amounts of contaminating proteins. The β -subunit band was excised from a preparative SDS-PAGE 5 gel and electroeluted, as described above.

The N-terminus of the beta subunit was blocked and therefore could not be sequenced. Internal amino acid sequence information was obtained by digesting the β -subunit with trypsin. The tryptic peptides were 10 resolved by reverse phase HPLC and eluted on a 60% acetonitrile gradient. Peaks analyzed by absorbance at 214 and 280 nm were collected and applied to a gas phase microsequenator.

The peptide sequences of two of these fragments 15 is:

P-61 Peptide Sequence:

LeuTyrGluAsnAsnIleGlnProIlePheAlaValThrSer

P-20 Peptide Sequence:

ThrAspThrGlyTyrIleGlyLys.

20 Two strategies were adopted for constructing oligonucleotide probes. A unique sequence 39mer was designed from peptide P-61 based on human codon usage frequency (Lathe, 1985, J. Mol. Biol. 183:1). Its sequence is:

25 3' - GACATACTCTTGTAGGTGGTAGAAACGACACTGG - 5'.

In addition, two sets of mixed sequence probes were constructed such that every possible sequence was represented. A 20mer of 96-fold redundancy was derived 30 from peptide P-61, and a 17mer of 192-fold redundancy was constructed based on the sequence from a different peptide fragment of the β -subunit, P-20. These sequences are given below.

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20mer, Mixed Sequence 3' - ATACTATTATTATAAGTCCC - 5'

G C G G C T
G

17mer, Mixed Sequence 3' - CTATGACCAATATAACC - 5'

5

G C C G G
G G T
T T

The 39mer and the mixed sequence 20mer were used to probe a Northern blot of poly A+selected RNA from PMA-activated U937 cells. The U937 cells, JY lymphoblastoid cells, HeLa cells, and CO3 cells (Springer et al., 1984, J. Exp. Med. 160:1901, an EBV-transformed cell line from a healthy donor) were grown in RPMI 1640 containing 10-15% fetal calf serum in a humidified atmosphere of 5% CO₂ at 37°C. The U937 cells were activated with 2 ng/ml PMA for three days prior to harvesting. The cells were lysed in a 4M guanidinium isothiocyanate solution, and RNA isolated in a 5.7M CsCl gradient. Poly A+ mRNA was selected with oligo (dT)-cellulose columns (Maniatis et al., Molecular Cloning: A laboratory manual, Cold Spring Harbor Laboratory, N.Y., 1982) or oligo (dT)-affinity paper (Amersham). This RNA was denatured and sized on a 1% agarose gel containing formaldehyde (Maniatis et al., supra) and transferred to nylon membranes (BioRad) in 20X SSC. A lane containing 28S and 18S ribosomal RNA from human cells or 23S and 16S rDNA from Escherichia coli was run to provide molecular weight standards.

The filters were hybridized with nick-translated probe DNA at 42°C for 18 hr in 5 X SSPE, 50% formamide, 10% dextran sulfate, 1 X Denhardts, 0.5% SDS and 100 ug/ml denatured salmon sperm DNA, and washed at high stringency (65°C) in 0.2 X SSC and 0.1% SDS. Both probes identified a band of approximately 3 kb.

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The 39mer gave a much stronger signal and was chosen for the primary screening of a cDNA library.

A human tonsil cDNA library (gift of L. Klickstein) was size-selected for inserts of 2kb or greater and constructed in ggt11 (Wong et al., 1985, Proc. Nat. Acad. Sci. U.S.A. 82:7711). The original library of 4×10^6 recombinants was amplified once, and 200,000 recombinants plated at a density of 7500 plaques/100mm plate. The plaques were amplified in situ 10 on duplicate nitrocellulose filters, as described by Woo (1979, Meth. Enzym. 68:389).

The oligonucleotide probes were labeled with 32 P-ATP using polynucleotide kinase. The filters were prehybridized for at least 2 hr at 42°C in 6 X SCC, 1 X 15 Denhardts, 0.5% SDS, 0.05% phosphate buffer, and 100 mg/ml of salmon sperm DNA. Hybridization with the 39mer was overnight at 42°C in prehybridization solution containing 20 mg/ml tRNA. The filters were washed at 53°C to 55°C with 6 X SSC, 0.1% SDS, and 0.05% phosphate 20 buffer. The damp filters were covered with plastic wrap and exposed to film with an intensifying screen. Phage that gave positive signals on duplicate filters were plaque purified and rescreened with the 39mer at a higher wash temperature (60°C) and with 20mer and 17mer 25 mixed sequence probes. 15 positive clones were picked.

Eight of the clones crossreacted with each other and gave positive signals with the 20mer mixed sequence probe and the independent 17mer mixed sequence probe. These clones were chosen for further analysis.

To confirm the identity of the cDNA clones, a 30 263 bp PstI/EcoRI restriction fragment which hybridized to the 39mer was subcloned into M13 vector and sequenced by the Sanger dideoxy chain termination method as follows. The amino acid sequence deduced from the DNA

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sequence is identical in 13 of 14 positions to the peptide sequence from which the 39mer probe was derived, including one amino acid which was not included in the design of the oligonucleotide. Furthermore, the 5 predicted amino acid sequence shows that this peptide is preceded by a lysine and followed by an arginine, as expected for a tryptic fragment. The one mismatch may be due to normal polymorphism. The unique sequence oligonucleotide was 87% homologous to the cDNA sequence, 10 despite the one amino acid mismatch.

The cDNA clones were restriction mapped by single and double restriction digests and, after end-labeling, by partial restriction digests (Maniatis et al., supra). Compatible restriction fragments were subcloned directly into M13 cloning vectors. Other 15 fragments were first blunt ended with Klenow, T4 polymerase, or Mung Bean nuclease (Maniatis et al., supra) and ligated into the HincII or SmaI site of the M13 polylinker. The nucleotide sequence of both strands was determined by the dideoxy chain termination method 20 of Sanger et al. (1977, Proc. Nat. Acad. Sci. U.S.A. 74:5463) using ³⁵S-dATP.

The complete nucleotide sequence and deduced amino acid sequence of the β -subunit gene in the longest clone, 18.1.1 (2.8 kb in length), is shown in Figure 1. 25 The first ATG is at position 73, and the sequence surrounding the ATG is consistent with the consensus rules for an initiation codon (Kozak 1984, Nucl. Acid. Res. 12:857). This putative initiation codon is followed by an open reading frame of 2304 bp, which 30 could encode a polypeptide of 769 amino acids (aa). The stop codon ATC is followed by a 3' untranslated region of 394 bp. The poly A tail was not found, although a

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consensus polyadenylation signal (AATAAA) is located 9 bp from the 3' end.

The deduced amino acid sequence of the cDNA clones was compared to peptide sequence data from the beta subunit of Mac-1, LFA-1, and p150,95 (Fig. 2). In addition to the P61 and P-20 peptide sequences given above, one other peptide was sequenced from the beta subunit of p150,95. Tryptic peptides were also prepared and analyzed from the beta subunit of purified Mac-1 and LFA-1. Each peptide sequence is found within the deduced amino acid sequence (Figs. 1 and 2). Thus, it can be concluded that the cDNA encodes the β -subunit of human LFA-1.

The cDNA clones hybridize to a single mRNA species of approximately 3.0 kb, which is the same message identified by the 39mer oligonucleotide. This message is present in PMA-activated U937 cells ($LFA-1^+$, $Mac-1^+$, $p150,95^+$), JY lymphoblastoid cells ($LFA-1^+$, $Mac-1^-$, $p150,95^-$), and EBV-transformed cells from a normal donor ($LFA-1^+$, $Mac-1^-$, $p150,95^-$), but is absent in HeLa cells ($LFA-1^-$, $Mac-1^-$, $p150,95^-$). Although clone 18.1.1 lacks the poly A tail, it is close to the estimated size of the RNA message.

Within the deduced polypeptide are two regions of sufficient length and hydrophobicity that could span the membrane bilayer. The first domain, which begins with the putative initiation methionine and extends 22 amino acids, has the characteristics of a signal sequence. This putative signal sequence is followed by a charged glutamine, a residue which is often cyclized at the N-terminal position. This would be consistent with the N-terminal blockage of the β -subunit, if the signal sequence is cleaved during processing.

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Use

The cDNA encoding the β -subunit of human LFA-1 can be used to produce recombinant β -subunit in large amounts. For example, the beta-subunit-encoding cDNA 5 can be excised from the ggt11 clones and introduced into an expression vector (plasmid, cosmid, phage or other type) to express the β -subunit in *E. coli*, using standard techniques. Alternatively the clones may be inserted into other vectors, such as mammalian, insect, 10 or yeast expression vectors, and used to produce recombinant β -subunit in mammalian or yeast cells.

The subunits produced by the above methods can be readily purified and used as an immunogen to raise monoclonal antibodies to the subunits. These antibodies 15 can be labelled and used in standard immunoassays to monitor the level of LFA-1, Mac-1, or p150,95 in white blood cells, and in the serum or other body fluids of patients having medical disorders associated with too many or too few cells having on their surfaces LFA-1 or 20 related proteins. For example, diseases, e.g., AIDS, characterized by immunosuppression can be expected to be accompanied by abnormally low levels of such cells, which are instrumental in fighting infections, and such diseases can thus be monitored by monitoring levels of 25 these proteins. Also, other disease states, e.g., autoimmune disease, allograft rejection, and graft-versus-host disease, can be expected to be characterized by abnormally high levels of such cells, and thus can also be monitored by monitoring levels of 30 these proteins. They can also be used to diagnose leukocyte adhesion deficiency, an inherited disorder caused by lack of LFA-1, Mac-1, and p150,95 glycoproteins. Antibodies to the β -subunit can also be

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used to purify LFA-1 or related proteins by conventional immunoaffinity purification methods.

The purified proteins, particularly LFA-1, Mac-1 and/or p150,95, whether native or recombinant, can also be used therapeutically. The proteins can be administered to patients in need of such treatment in an effective amount (e.g., from 20-500 mg per kg body weight), and mixed with a pharmaceutically acceptable carrier substance such as saline. Therapeutic utility of these proteins is based on the fact that disease states such as autoimmune diseases, allograft rejections, and graft-versus-host diseases involve abnormally high levels of cell-to-cell contact mediated by the recognition and binding of LFA-1 and related proteins to target antigen presenting cells, endothelial cells, and other types of cells. The administration of LFA-1 or a related protein, or fragments thereof, will compete for receptors for the cell-bound protein, inhibiting cell-to-cell binding and thus bringing about the desired immunosuppression. A particular disease for which these proteins will be useful is the autoimmune disease rheumatoid arthritis. Preferably administration is intravenous at about 20-500 mg per kg body weight, or directly at an inflamed joint of a patient suffering from rheumatoid arthritis. Alternatively, oral administration or local application can be used by providing tablets, capsules, or solutions, or by applying lotions as required. The amount and method of administration will vary dependent upon the age and weight of the patient, and the disease to be treated. Other autoimmune diseases which can be treated include systemic lupus erythematosis, juvenile onset diabetes, multiple sclerosis, allergic conditions, eczema, ulcerative colitis, inflammatory bowel disease, Crohn's

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disease, as well as allograft rejections (e.g.,
rejection of a transplanted kidney or heart). LFA-1,
Mac-1, and p150,95 normally act in situ by binding to
endothelial and other cells. Thus, the free proteins or
5 peptides, which are administered, will be able to
inhibit leukocyte immune responses and migration to
inflammatory sites.

The β subunit cDNA clone can be used in
prenatal diagnosis of leukocyte adhesion deficiency
10 (LAD). LAD disease is a deficiency in cell surface
expression of LFA-1, Mac-1, and p150,95 and is due at
least in part to a primary genetic lesion in the β
subunit. Patients with the severe form of LAD disease
suffer from recurrent bacterial infections and rarely
15 survive beyond childhood. The defect can be detected
early in pregnancy since it is associated with a unique
restriction fragment length polymorphism. PstI
digestion of human DNA and hybridization with the 1.8 kb
EcoRI fragment (shown in Fig. 2) of the β subunit cDNA
20 defines a restriction fragment length polymorphism
(RFLP). Diagnosis of this disease is therefore
performed by standard procedure using the whole or a
part of this EcoRI fragment. The genomic DNAs of the
parents of the fetus, and the fetus are screened with
25 this probe and an analysis of their RFLPs made. In this
way the probability that the fetus has the disease can
be estimated.

Other embodiments are within the following
claims.

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Claims

1. A cDNA sequence encoding a) the β -subunit of a human glycoprotein concerned with cellular adhesion, b) a biologically active fraction of said glycoprotein, c) an analog of said glycoprotein, or d) a fragment of said glycoprotein comprising at least 10% of a contiguous sequence of said cDNA.
2. A vector comprising a DNA sequence encoding the β -subunit of a human glycoprotein concerned with cellular adhesion, or a biologically active fraction thereof, or an analog thereof, or a fragment thereof encoding at least 10% of a contiguous sequence of said β -subunit.
3. Substantially pure recombinant β -subunit of a human glycoprotein concerned with cellular adhesion, or a biologically active fraction thereof, or an analog thereof, or a fragment thereof comprising at least 10% of a contiguous sequence of said subunit.
4. A monoclonal antibody raised against recombinant β -subunit of a human glycoprotein concerned with cellular adhesion, or a biologically active fraction thereof, or an analog thereof, or a fragment thereof comprising at least 10% of a contiguous sequence of said subunit.
5. The cDNA of claim 1 wherein said glycoprotein is LFA-1, Mac-1 or p150,95.
6. The vector of claim 2 wherein said glycoprotein is LFA-1, Mac-1 or p150,95, and said fragment comprises at least 80% of a contiguous sequence of said DNA.
7. The β -subunit of claim 3 wherein said glycoprotein is LFA-1, Mac-1, or p150,95, and said fragment comprises at least 80% of a contiguous sequence of said DNA.

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8. The antibody of claim 4 wherein said glycoprotein is LFA-1, Mac-1, or p150,95, and said fragment comprises at least 80% of a contiguous sequence of said DNA.

5 9. The cDNA sequence of claim 1, said sequence being substantially the same as at least 10% of the DNA sequence shown in Figure 1.

10. A method of treating an animal suffering from a medical condition characterized by an undesirably high level of leukocyte interaction with other cells, comprising administering to said patient an amount of LFA-1, Mac-1, or p150,95, or an effective fragment thereof, wherein said amount is effective to minimize said leukocyte interaction.

15 11. A method of monitoring the level of glycoproteins in an animal comprising assaying a body fluid of said patient for LFA-1, Mac-1, or p150,95, wherein said assaying comprises detecting said LFA-1, Mac-1 or p150,95 with antibodies produced to recombinant 20 said glycoproteins.

12. A recombinant vector comprising at least a contiguous 10% section of the DNA sequence shown in Fig. 1.

13. A method for diagnosing leukocyte adhesion deficiency comprising digesting human DNA with a restriction enzyme, probing said DNA with a probe specific for a restriction fragment length polymorphism associated with said deficiency, and observing the length of a restriction fragment hybridizing to said 30 probe; wherein the length of said restriction fragment is diagnostic of said disease.

14. The method of claim 14 wherein said probe is an 1.8 kb EcoRI fragment encoding the β -subunit of human LFA-1, and said restriction enzyme is Pst-I.

Fig. 1 (page 11)

CAGGGAGCTGTGACCA GCCCCAGCCAGGAGGA GCAACGGCGGATTCCAGC ACACCGGAACTATG ATG CTC GGG CCC CCA CTC CTC GCC CTC GTC GGG CTC CTC ICC CTC
HET Leu Gly Leu Arg Pro Pro Leu Ala Leu Val Gly Leu Ser Leu 17

124 GGG TGC GTC CTC TCT CAG GGG TGC AAG TTC AAG GTC AGC AAG TGC CTC GAA TGC AAG TGG CTC ACC TGG TGC CAG AAG CTG AAC TTC ACA GGG
Gly(Cys)Val Leu Ser Glu(Cys)Thr Lys Phe Lys Ser Cys(Arg Glu(Cys))Ile Glu Ser Gly Pro Gly(Cys)Thr Trp(Gly)lys. Leu Asn Phe Thr Gly 53

232 CGG GGG GAT CCT GAC TCC ATT CGC TGC GAC ACC CGG CCA CAG CTC CTC ATG AAG GGC TGT GCG GTT GAG GAC ATG GAC AAG CTC GCT GAA ACC CAG GAA
Pro Gly Asp Pro Asp Ser Ile Arg(Cys)Asp Thr Arg Pro Glu Leu HET Arg Gly(Cys)Ala Ala Asp Ile HET Asp Pro Thr Ser Leu Ala Glu Thr Glu Glu 89

340 GAC CAC AAT GGG GGC CAG AAG CAG CTC TCC CCA AAA GTG AGG CCT TAC CTG CCA CCA GGC CAG GAA GCA GGG TTC AAC GTG ACC TTC CGG CGG CCG AAG GGC TAC
Asp His Asn Gly Gly Glu Lys Glu Lys Glu Leu Ser Pro Glu Lys Val Thr Leu Tyr Pro Gly Glu Ala Ala Ala Phe Asn Val Thr Phe Arg Arg Ala Lys Gly Tyr 125

448 CCC ATC GAC CTC TAC TAT CTC AIG CTC TAC CTC AAG CTC AGG ATT GAT GAC CTC AAG AAG CTA GGT GGC GAC CTC CGG CGG CTC AAC GAG ATC ACC
Pro Ile Asp Leu Tyr Leu HET Asp Leu Ser Tyr Ser HET Leu Asp Asp Leu Asn Val Lys Lys Leu Glu Gly Asp Leu Leu Arg Ala Leu Asn Glu Ile Thr 161

556 GAG TCC GGC CGC ATT GGC TTC GGG TCC TTC GTC GAC AAG ACC GTC AAC AGC CAC CCT GAT AAG CTC CGA AAC CCA TGC CCC AAC AAG GAG AAA GAG
Glu Ser Gly Arg Ile Gly Phe Gly Ser Phe Val Asp Lys Thr Val Leu Pro Phe Val Asn Thr His Pro Asp Lys Leu Arg Asn Pro(Gly)Pro Asn Lys Glu Lys Glu 197

664 TGC CAG CCC CTT GGC TTC AGG CAC GTC AAG CTC ACC AAC AAC TCC AAC CAG TTT CAG ACC GAG GTC GGG AAG CAG Gln Thr Glu Val Gly Lys Glu Ile Ser Gly Asn Leu Asp Ala Pro
(Cys)Gln Pro Pro Phe Ala Phe Arg His Val Leu Lys Leu Thr Asn Ser Asn Glu Phe Glu Ile Ser Gly Asn Leu Asp Ala Pro 223

772 GAG GAT GGC CTG GAC GGC AGG ATG CAG GTC GGC TGC TGC GAG GAA ATC GGC TGG CGG AAC GTC AGG CGG CTC ACT GAT GAC GGC TTC CAT TIC
Glu Gly Gly Leu Asp Ala HET Glu Val Ala (Cys)Pro Glu Glu Ile Gly Trp Arg Asn Val Thr Arg Leu Leu Val Phe Ala Thr Asp Asp Gly Phe His Phe 269

1-56a
H-5b

Fig. 1 (continued page 2)

880	GCG GGC GAC GGA AAG CTG CGC GCC ATC CCC AAC GAC GGC CGC TGT CAC CTG GAG GAC AAC TTG TAC AAG AGG AGC AAC GAA TTC GAC TAC CCA TCG TGT GGC Ala Gly Asp Gly Lys Leu Gly Ala Ile Leu Thr Pro Asn Asp Gly Arg(Cys)His Leu Glu Asp Ser Asn Glu Phe Asp Tyr Pro Ser Val Gly	305
988	CAG CTG GCG GAC AAC CTG GCT GAA AAC ATC CAG CCC ATC TTC CGG GIG ACC AGT AGG ATG GTG AAG ACC TAC TAG AAA CTC ACC GAG ATC ATC CCC AAG TCA GCC Gln Leu Ala His Lys Leu Ala Glu Asn Ile Glu Asn Asn Ile Phe Ala Val Thr Ser Arg Met Val Lys Thr Tyr Glu Lys Leu Thr Glu Ile Pro Lys Ser Ala	341
1096	GTG GGG GAG CTG TCT GAG GAC AAC ATG GAT GCT TAC AAT AAA CTC TCC AEG GTC CTG GAT CAC AAC GCC CTC CCC GAC ACC Val Gly Glu Leu Ser Glu Asp Ser Ser Asn Val Val His Leu Ile Lys Asn Ala Tyr Asn Lys Leu Ser Ser Arg Val Phe Leu Asp His Asn Ala Leu Pro Asp Thr	377
1204	CTG AAA GTC ACC TAC GAC TCC TIC TGC TGC AGC AAC GAG CAC AGG AAC GAG CCC AGA GGT GAC TGT GAT GGC GTC CAG ATC AAT GTC ECG ATC ACC TTC CAG GTG Leu Lys Val Thr Tyr Asp Ser Phe(Cys)Ser Asn Gly Val Thr His Arg Asn Gln Pro Arg Gly Asp(Cys)Asp Gly Val Glu Ile Asn Val Pro Ile Thr Phe Gln Val	413
1312	AAG GTC ACG GCC ACA GAG TGC ATC CAG TGG TTT GTC ATC CGG CGG CGC TTC AGC GAC ATA GTC ACC GTC CAG GTT CTT CAG TGT GAG TGC CGG TGC Lys Val Thr Ala Thr Glu(Cys)Ile Glu-Gln Ser Phe Val Ile Arg Ala Leu Gly Phe Thr Asp Ile Val Thr Val Gln Val Val Pro Glu(Cys)Glu(Cys)Arg(Cys)	449
1420	CGG GAC GAG AGA GAC GGC CTC TGC CAT GGC AAG GGC TTC TIG GAG TGC GGC AGG TGT GAC ACT GGC TAC ATT GGG AAA AAC TGT GAG TGC CAG ACA Arg Asp Gln Ser Arg Asp Arg Ser Leu(Cys)His Gly Lys Gly Phe Leu Glu(Cys)Gly Ile(Cys)Arg(Cys)Arg(Cys)Asp Thr Gly Tyr Ile Gly Lys Asn(Cys)Glu(Cys)Gln Thr	485
1528	CAG GGC CGG AGC AGC CAG CGG CTG GAA GGA AGC TCC CGG AAG GAC AAC TCC AIC AIC TCC AIC AIC TGC TCA EGG CTG GGG GAC TGT GTC TCC CAG TGC TGC CAC ACC Gln Gly Arg Ser Ser Gln Glu Leu Glu Gly Ser(Cys)Arg Lys Asp Asn Asn Ser Ile Ile(Cys)Ser Gly Ile Gly Asp(Cys)Val(Cys)Gly Glu(Cys)Ile(Cys)His Thr	521

fig. 1 (continued page 3)

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FIGURE 2

p150,95 β Subunit

P-61 sequence	L Y E N N I Q P I F A V T S
Deduced sequence	K L A E N N I Q P I F A V T S
P-20 sequence	(T/C) D T G Y I G K
Deduced sequence	R C D T G Y I G K
P-18 sequence	S S Q E L E G S (T/C) (R)
Deduced sequence	R S S Q E L E G S C R

Mac-1 β Subunit

M-58 sequence	L L V F A T D D G F H F
Deduced sequence	R L L V F A T D D G F H F
M-52 sequence	X A V G E L S E X(S) X N
Deduced sequence	K S A V G E L S E D S S N

LFA-1 β Subunit

L56a sequence	E C Q P P F A F R
Deduced sequence	K E C Q P P F A F R
L56b sequence	L I Y G Q Y C E(C) D T I
Deduced sequence	K L I Y G Q Y C E C D T I
L-65 sequence	V F L D H N A L P
Deduced sequence	R V F L D H N A L P

INTERNATIONAL SEARCH REPORT

International Application No. PCT/US88/00611

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) ⁶

According to International Patent Classification (IPC) or to both National Classification and IPC
 IPC(4): C07H 21/04; C12N 15/00

II. FIELDS SEARCHED

Minimum Documentation Searched ⁷

Classification System	Classification Symbols
U.S.	435/68, 70, 71, 91, 172.1, 172.3, 243, 253, 320; 536 /27 530/324, 325, 837; 935/9, 10, 11, 22

Documentation Searched other than Minimum Documentation
to the Extent that such Documents are Included in the Fields Searched ⁸

CA FILE 1967-1988
 BIOSIS FILE 1967-88

III. DOCUMENTS CONSIDERED TO BE RELEVANT ⁹

Category ¹⁰	Citation of Document, ¹¹ with indication, where appropriate, of the relevant passages ¹²	Relevant to Claim No. ¹³
Y	T.A. SPRINGER ET AL , "Sequence homology of the LFA-1 and Mac-1 leukocyte adhesion glycoproteins and unexpected relation to leukocyte interferon", Nature, Volume 314, pages 540-542, published 11 April 1985 by MacMillan Journals LTD (London, UK). See especially pages 540 and 541.	1, 2, 5 and 6
Y	F. SANCHEZ-MADRID ET AL , "Mapping of antigenic and functional epitopes on the -and subunits of two related mouse glycoproteins involved in cell interactions, LFA-1 and MAC-1", The Journal of Experimental Medicine, Volume 158, pages 586-602, published August 1983 by the Rockefeller University Press (New York, NY, USA). See especially pages 591-594.	1, 2, 5 and 6

* Special categories of cited documents: ¹⁰

- "A" document defining the general state of the art which is not considered to be of particular relevance
- "E" earlier document but published on or after the international filing date
- "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- "O" document referring to an oral disclosure, use, exhibition or other means
- "P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search

18 JUNE 1988

Date of Mailing of this International Search Report

19 JUL 1988

International Searching Authority

ISA/US

Signature of Authorized Officer


 JAMES MARTINELL

FURTHER INFORMATION CONTINUED FROM THE SECOND SHEET

V. K. KURZINGER ET AL, "Structural homology of a macrophage differentiation antigen and an antigen involved in T-cell mediated killing", Nature, Volume 296, pages 668-671, published 15 April 1982 by MacMillan Journals LTD (London, UK). See entire document.

1,2,5
and 6

V. OBSERVATIONS WHERE CERTAIN CLAIMS WERE FOUND UNSEARCHABLE¹

This international search report has not been established in respect of certain claims under Article 17(2) (a) for the following reasons:

1. Claim numbers _____ because they relate to subject matter¹² not required to be searched by this Authority, namely:

2. Claim numbers 9 and 12, because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out¹³, specifically:

Claims 9 and 12 refer to Figure 1, which figure is not completely legible. See Article 17(2)(a)(ii) and 17 (2) (b).

3. Claim numbers _____, because they are dependent claims not drafted in accordance with the second and third sentences of PCT Rule 6.4(a).

VI. OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING²

This International Searching Authority found multiple inventions in this international application as follows:

I. Claims 1,2,5,6,9 and 12.

VI. Claims 13 and 14.

II. Claims 3 and 7.

III. Claims 4 and 8.

IV. Claim 10.

V. Claim 11.

1. As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims of the international application.

2. As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims of the international application for which fees were paid, specifically claims:

3. No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claim numbers:

1,2,5,6,9 and 12

4. As all searchable claims could be searched without effort justifying an additional fee, the International Searching Authority did not invite payment of any additional fee.

Remark on Protest

- The additional search fees were accompanied by applicant's protest.
- No protest accompanied the payment of additional search fees.